incidence precludes any definite conclusion. The number of animals used is also small for rigorous statistical analysis.

Szmigielski et al (1982, 1988) reported that exposure of mice to 2450 MHz microwaves at 2 - 3 or 6 - 8 W/kg, for 2 h per day for 6 days per week, for varying times up to 10 months; (1) accelerated the appearance of spontaneous mammary cancer, (2) decreased the time to occurrence of skin tumours induced by a carcinogen (3, 4-benzopyrene), and (3) increased the number of neoplastic nodules developing in the lungs of mice injected with cancer cells and examined 14 days later. These results suggest that RF radiation accelerated the development of three different types of tumours.

In a promotion study (Szmigielski et al 1988) mice were exposed to SARs of between 2 and 8 W/kg for 2 h per day for some months following the injection of 2×10^5 sarcoma cells. The development of cancerous nodules in the lung showed a dose-dependence. However, at an exposure of 2 - 3 W/kg the effect was comparable with that which can occur from chronic non-specific stress. It has been suggested by the authors that impaired immune surveillance resulted in a lowering of resistance to neoplastic growth. The skin tumour study reported an increased number of tumours when microwave radiation followed a sub-carcinogenic dose of benzopyrene.

The results of a study of 100 rats exposed for most of their lifetime at about 0.4 W/kg (Chou et al 1992) have created some disagreement in interpretation. The exposure consisted of 2.45 GHz frequency pulsed (800 pps, 10 μ pulse width) waveform modulated at 8 Hz. The whole body average SAR was estimated to range from 0.4 to 0.15 W/kg for rats weighing 200 to 800 gm, respectively. Rats were exposed for 21.5 h per day from 8 weeks of age for 25 months (i.e. lifetime). There was some evidence of effects on corticosterone level after 13 months, that was not repeatable in a smaller follow-up study. The important result was a statistically significant increase in incidence of primary malignancies in the exposed (18) compared to control (5) group. The numbers of such spontaneous cancers are too low to achieve statistical significance when classified according to each type. There is a developing opinion that a single toxic agent may be capable of producing more than one type of tumour.

Comments on the protective role of melatonin, Prof. Reiter (University of Texas, Health Science Centre, San Antonio) has been quoted as saying that the

suppression of melatonin by magnetic fields could result in a higher incidence of cancer in any tissue, and therefore a wide range of different tumour types.

5.3 COMMENTS ON CANCER-RELATED STUDIES

If EMR is suspected of being involved in cancer development there are a number of issues that need to be tested:

(a) What cellular evidence exists, such as altered DNA, RNA, transcription rate, colony forming efficiency?
(b) Is there evidence of cell membrane changes resulting in altered ionic distribution?
(c) Is there evidence of immune response impairment, that may allow tumour development to proceed?
(d) Is there *in vivo* evidence of an increased incidence of tumour growth in laboratory animals or in humans?

It is virtually impossible to detect effects with epidemiology surveys without prior knowledge of the most likely biological effect. Teratologists now acknowledge that unless the full spectrum of abnormalities is known there is a very small chance of detecting even a strong teratogenic compound by epidemiology. For example, a drug such as thalidomide, which can produce a wide range of limb deformities can only be identified with certainty if all reports of every expected deformity from digital amputations to absence of one or more limbs is included. The use of a specific single effect would preclude its detection. To detect, with any degree of certainty, the effects of EM radiation, information is required on mechanisms of interaction to predict the likely consequences.

The use of genetically compromised animals that are predisposed to a certain type of cancer may be inappropriate as the high base level incidence may mask small differences from the mean value thereby reducing its statistical power. Past chronic exposure animal studies have produced conflicting results, with one study (Chou et al 1992) giving either; (a) a positive or (b) a negative result depending on whether one interprets a real effect as, (i) an increased incidence in all cancers in the population, or (ii) an increased incidence of a specific cancer.

The studies currently undertaken by Adey's group represent the most thoroughly controlled chronic exposure protocol employed to date, and are being carried out at a frequency relevant to cellular telephones. Results will be available in 1995,

although it will be some time later before a publication appears. Whatever the result, it will at best represent a starting point for subsequent careful study. It is most important that new studies learn from this experience.

Rigorous animal experimental studies are demanding, expensive and require dedicated attention to detail for extended periods. The appropriate combination of specialised facility and trained staff is a rare commodity. Carefully worked protocols using sufficiently large exposed, sham-exposed and control groups are essential to achieve a valid evaluation of this effect. Small scale experiments comparing groups of less than 100 have low statistical power and therefore contribute little to the debate. Similarly, studies which irradiate animals with rapidly growing brain tumours are confounded by the premature death of the animal. Thus, the consequences of long term exposure to low level radiation are not tested. A recent presentation (Salford et al 1993) is such an example, where a total of 62 rates were irradiated with 915 MHz at six different exposure regimes for 7 h per day until death. Each animal had been inoculated with a large dose of glioma cells at five days prior to irradiation and died as a result of rapid growth of a large brain tumour.

True scientific protocol requires the establishment of an hypothesis which must be repeatedly tested before any inference can be drawn from the results. The *in vitro* cell studies have provided some clues about setting such hypotheses. Perhaps the most important were the experiments of Cleary et al (1990 a) which demonstrated an altered rate of DNA synthesis and proliferation of human glioma cells after a single exposure to microwave radiation. This abnormal behaviour is consistent with early changes seen in cells that lead to tumour formation. Effects were observed at both 27 and 2450 MHz frequency and with cw or pulsed waveforms. Furthermore, he also reported the effect in cultured human glioma cells (Cleary et al 1990 b). The exposures were applied over a range of SAR, with the lowest level at which the effect was observed as 5 W/kg. Although the exposure conditions have been reported as non-thermal it is difficult to see how the exposure could avoid large thermal gradients from the cells to the cooling fluid surrounding the cell culture vessel.

What makes these studies so interesting is that the effect occurs after a single 2 h exposure and lasts for up to five days. Thus, a daily exposure regimen would reinforce the effect. The connection between accelerated growth of human brain tumour cells in culture to that occurring *in vivo* during repeated exposure to

EMR is one that deserves close examination. Hence the need for data from chronic animal studies and human epidemiology surveys. The extrapolation of results from laboratory rodents to humans is always fraught with difficulties and divergent opinions.

Relevance to Cellular Telephones

The public concern about cellular telephones and cancer exists because of reports of an association between extremely low frequencies (ELF; 50 or 60 Hz) or police radar and cancer, and because of a lawsuit about cellular telephone use and a case of parietal lobe glioblastoma. The distinction between ELF and RF is rarely made. Reports exist of brain cancer and leukemias in epidemiology studies of nonionizing radiation at frequencies different from those emanating from cellular telephones. Little is known of human experience with cellular telephones because: (a) there are no epidemiology studies at this frequency; (b) the amount and distribution of absorption is not fully known; and (c) brain cancer is a rare event (less than 1% incidence) with a long latency period. Experimental animal studies have not been described at 915 MHz. However, a study of rats exposed to 2450 MHz radiation reported a 3-fold increase in primary malignant neoplastic lesions. Studies of different cell lines at 27 or 2450 MHz frequencies demonstrated a dose-dependent increase in growth rate that persisted for about five days, after a single 2 h exposure.

Review of the literature on radiofrequency radiation and cancer yields results from eight animal studies over a 30 year period. Several of the studies reported increases of tumour incidence in the irradiated groups, especially mammary tumours and leukemias. None of these studies, used cellular telephone frequencies. National Toxicology Program (NTP) studies of chemical carcinogenesis in rodents exhibit a correlation between mammary tumours or leukemias and decreased life span. Because radiofrequency radiation doses that did not increase body temperature had no effect on life span the hypothesis has been proposed of no positive correlation between radiofrequency radiation and cancer induction.

For any biological effect to become significant the body's homeostatic mechanism has to be overcome. Homeostasis uses cellular communications via molecules and ions to control the three basic functions of cells: proliferation, differentiation, and activation. Cancer promotion involves the disruption of cell-to-cell communication. One way that this can occur is through the closure of

gap junctions between the cells. This disruption is both reversible and dose-responsive. If the promoter is removed, intercellular communication returns to normal. With chemical carcinogenesis, the promoting agent must be applied at a high dose, constantly, over a long period of time. During carcinogenesis a normal cell is transformed into a neoplastic cell *(initiation)*, and the neoplastic cell grows into a neoplasm *(promotion)*. Cell proliferation is important for both steps, and stimulation of cell proliferation would sensitise cells to the effects of endogenous DNA damage that occurs spontaneously. This is one pathway for epigenetic carcinogenesis. Thus, a carcinogenic agent does not have to alter DNA directly. An initiating agent may need only a single exposure to alter the genome and induce cancer, while promotion requires high doses, is reversible, and must continue for a long duration. Therefore, the risks from promotion are less than the risks of initiation.

In general, the energy level of a cellular telephone is not sufficient to break chemical bonds. Thus, nonionizing radiation is not likely to *initiate*, because it cannot directly induce alterations in the genome. Except for pulsed field, cell membranes absorb much of the radiation and are a target for nonionizing radiation, and for chemical agents that act as cancer *promoters*. Effects at the cell membrane are consistent with promotion. Because nonionizing radiation can induce ornithine decarboxylase, the mechanism of carcinogenesis would be more likely to be epigenetic.

Conclusion

Currently there are neither data that cellular telephone use induces cancer in humans, nor any data that link nonionizing radiation from cellular telephones to tumour induction in animals. The significance of some studies showing tumour promotion is uncertain.

Recommendations

If we consider the possible promoting effects of EMR on the development of cancer then the most relevant studies would be those carried out in animals exposed to low level radiation for extended durations. To be reliable, these studies need to use a sufficiently large population to be statistically powerful and must be designed in such a way that all known confounding variables are controlled. A major difficulty in conducting long-term exposure experiments is the problem of ensuring properly controlled exposures to large numbers of animals. It is essential to provide both an acceptable, low stress, living

environment for the animals and a uniform microwave exposure for the entire population. RF coupling to the animals body will differ as a function of number of animals in the group, distance between them, and orientation of each animal in the RF field (D'Andrea & de Lorge 1990). At the same time, there is good reason to have concurrent exposure to normal environmental pollutants that might potentiate the effects. Finally, the protocol needs to be robust and repeatable to allow exact duplication of the study in an independent laboratory.

It is well known that the distribution of RFR in an exposed object depends on many factors including frequency, orientation of exposure, dielectric constant of the constituent tissue. The design of experimental protocols is critical if the results are to provide meaningful extrapolation to a particular RF source. Cellular telephones are used in a specific manner. Most people would hold a phone to the same ear in the same orientation and proximity to the skull. Usually one would expect the antenna to be close to the parietal bone (although many airport officials have a peculiar habit of holding the large portable phones in front of their mouth so that they look across the top of the antenna). However, assuming normal usage patterns it would make sense to design experiments so that the RF source was located towards the lateral aspect of the skull. Chou et al (1985a) found significant differences in local SARs in eight different regions of the brain of rats and these all changed in each of seven different exposure arrangements. Lai et al (1984a) reported a difference in microwave response with pentobarbital depending on whether the rat was facing toward or away from the source of irradiation in a waveguide when the average whole body SAR remained constant; patterns of energy absorption in the brain differed substantially.

6.0 GENETIC EFFECTS

SUMMARY

The majority of studies show that exposure to RF radiation does not result in an increase in chromosome aberration frequency when temperatures are maintained within physiological limits. It is well known that hyperthermia induces profound alteration in gene expression as demonstrated by the heat-shock response in early post-implantation rodent embryos (Walsh et al 1985). The so-called heat-shock response is elicited by a wide range of irritants and stresses apart from heat. There is evidence of a synergistic effect when embryos are exposed to pulsed ultrasonic energy combined with a modest increase in bulk heating (Angles et al 1990). Potentiating effects of non-ionizing radiation by environmental factors require investigation.

Reported increased frequency of cytogenetic effects after *in vivo* exposure to 2.45 GHz at SAR up to 20 W/kg was not successfully replicated in a study using a different strain of mouse. Reported increases in the frequency of sister chromatid exchanges following *in vitro* exposure have not been verified.

The literature contains disagreement on the effect of microwave radiation on chromosome aberration frequency in male germ cells.

An indicator of altered chromosome aberrations, increased dominant lethality (assessed as impaired survival of implanted embryos), was reported after acute exposure to high power levels where hyperthermic effects were dominant. There is no evidence of induced dominant lethals in rodents exposed to SAR up to 5 W/kg in chronic exposures over periods up to 8 weeks. While this is interesting, its use in establishing health risk is somewhat limited. To be meaningful for human health implications, it is essential that these studies are conducted in a manner that is relevant to environmental exposures of EMR. Humans are exposed to low level EMR from prenatal existence throughout life and it is appropriate that animal models should at least be exposed to similar conditions if the scientific data base is to be improved.

Introduction

The potential interference by EMR on the structure of DNA or chromosomes is an important consideration in somatic cells where a non-lethal change could be associated with the development of cancers. If such effects occur in the male or

female germ cells, surviving mutations might be passed on to subsequent generations. These effects are conveniently studied in cell culture and small animal exposures where relatively sensitive tests can assess the rate of change in single and multiple generations.

The possibility of adverse effects of RF radiation on male germ cells has received media attention recently in the USA where claims that RF emissions from public radar guns may be responsible for an increase in the incidence of testicular tumours. It is not certain whether effects on sperm cell integrity were also considered.

6.1 Experimental Evidence

Studies on the possible hereditary interference by RF exposure are summarised in table 6.1. No confirmed adverse effects assessed by structural aberrations and sister chromatid exchanges were reported following *in vivo* exposures (Huang et al 1977; McRee et al 1981).

The literature contains disagreement on the effect of microwave radiation on chromosome aberration frequency in male germ cells. Original experiments exposing germ cells in male mice to microwave radiation (Manikowska et al 1979, 1985) reported an SAR dependant increase in the frequency of chromosome aberrations that has not been confirmed in subsequent studies when the rectal temperature increased by up to 3°C above the normal basal value (Beechey et al 1986). A further study exposed the spermatogonial stem cells (greatest risk of accumulating genetic damage) and found no evidence of altered frequency of chromosome translocations or fragments after *in vivo* exposures to 5 W/kg for a total of 120 h over a period of 8 weeks (Saunders et al 1988). There was no change in rectal temperature in exposed and sham-exposed mice. Temperature in the germ cells was not reported.

A number of studies has assessed the ability of microwave radiation to induce dominant lethal mutations which result in pre-implantation death or subsequent embryonic or fetal mortality. Contradictory results have been reported (table 6.1). Whilst one study (Varma & Traboulay 1977) reported an increase in induction of dominant lethal mutations after exposure to 1.7 GHz at 500 W/m^2 (SAR estimated as 25-45 W/kg), this was not replicated in two subsequent studies. The first of which exposed mice to 2.4 GHz at either 600 W/m^2 for 12 min or 8,000 W/m^2 for 21 s (Ramaiaya et al 1980) without observing an effect, although up to 10% of the mice died from the severity of the exposure. The second study found no evidence of increased dominant lethality

Table 6.1 Summary of studies on Genetic Bioeffects of EMR

Exposure conditions	Effect	Reference
2.45 GHz (cw) SAR to 21 W/kg *(in vivo)*	Chinese hamster: No increase in unstable chromosome aberrations in lymphocytes, after 72 h in culture Rectal temperature increased by 1.6°C	Huang et al 1977
2.45 GHz (cw) SAR 21 W/kg 8 h per day x 28 days	Mouse: No increase in sister chromatid exchange rate in bone marrow cells	McRee et al 1981
9.4 GHz (pulse modulated) 1-100 W/m^2 1 h per day x 14 days	Balb/C mice: Increased frequency of chromosome exchanges (SAR-dependent) Abnormalities in male germ cells when exposed as spermatocytes	Manikowska et al 1979
2.45 GHz (cw) 0.05-20 W/kg 6 h total over 14 days	CBA mice: Increased frequency of chromosome exchanges (SAR-dependent) Abnormalities in male germ cells exposed as spermatocytes.	Manikowska-Czerska et al 1985
2.45 GHz (cw) 0.05-20 W/kg 6 h total over 14 days	C3H mice: Frequency of chromosome aberrations not statistically different between control & exposed groups Rectal temperature rose by up to 3°C	Beechey et al 1986

Table 6.1 Summary of studies on Genetic Bioeffects of EMR

Exposure conditions	Effect	Reference
1.7 GHz (cw) 500 W/m^2, SAR 25-45 W/kg 0.5 h SAR 5-9 W/kg 0.66 h total during 2 week period	Swiss mice: (anaesthetised) Induced dominant lethal mutations Inconclusive evidence of mutagenicity Dosimetry questionable	Varma & Traboulay 1977
2.4 GHz (cw) 600 W/m^2 for 0.2 h 8,000 W/m^2 for 21 s	Mice: No evidence of increased dominant lethality	Ramaiaya et al 1980
2.45 GHz (cw) SAR 43 W/kg 0.5 h	Mice: No evidence of increased dominant lethality Pregnancy rate significantly reduced, probably due to heat-induced reduced sperm count	Saunders et al 1983
2.45 GHz (cw) 100 W/m^2, SAR 5 W/kg 6 h per day x 8 weeks total 120 h	Mice: No increased rate of chromosome abnormalities No evidence of induction of dominant lethality No significant reduction in pregnancy rates or pre-implantation survival No increase in rectal temperature	Saunders et al 1988

Table 6.1 Summary of studies on Genetic Bioeffects of EMR

Exposure conditions	Effect	Reference
2.45 GHz (cw) 50 W/m^2, SAR = 0.9-4.7 W/kg 4 h per day from gestation day 6 to 90 days postnatal.	Rats: No consistent pattern of responses	Berman et al 1980
100 W/m^2, SAR 2 W/kg 5 h per day x 5 days exposed as young adults	Increased fetal mortality, but not related to decrease in live fetuses	
280 W/m^2, SAR 5.6 W/kg 4 h per day x 5 days per week for 4 weeks	No evidence of male germ cell mutagenesis	
2.45 GHz (pulsed 50 Hz, duty cycle 1:3) SAR 75 W/kg	Human: Lymphocytes Increased frequency of chromosome aberrations	Maes et al 1993

in mice exposed to SAR 43 W/kg (Saunders et al 1983). However, significant reductions in pregnancy rate were observed which was subsequently attributed to impaired male fertility resulting from decreased sperm counts and an increase in the proportion of abnormally shaped sperm (Kowalezuk et al 1983). This is hardly surprising when one considers the likelihood of substantial heating of the testes from the absorbed dose.

It is interesting that previous studies apparently did not consider the possibility of impaired sperm cell function which is known to be sensitive to elevated temperature. Beechey et al (1986) reported an increase in rectal temperature of 3°C in mice exposed to 20 W/kg, while Saunders et al (1988) reported no change in temperature at 5 W/kg in mice exposed at the same 2.45 GHz frequency.

Chronic, low level exposures are more relevant to public health risk of electromagnetic radiations. There is no evidence to date of the induction of dominant lethal mutations in mice (Saunders et al 1988) or rats (Berman et al 1980). However, the longest term of these experimental exposures was 8 weeks which is rather short, even in relation to the life span of rodents.

Studies have been carried out using standardised techniques for clastogenic and cytogenic effects following *in vitro* radiation with microwaves. Effects on cell cycle kinetics and cell proliferation rates are endpoints that would be expected to show a response to cellular perturbation. Elevated temperature is a common cause of altered rates of mitotic division.

Although the generally-held opinion is that the energy levels available in non-ionizing radiation are insufficient to cause chromosome breaks a recent study reported a statistically significant increase in the frequency of chromosome aberrations and micronuclei (Maes et al 1993) in human peripheral blood lymphocytes. A temperature controlled "normothermal" exposure system exposed lymphocytes *in vitro* in a tube containing a thermistor to regulate the microwave output and maintain a constant temperature of 36.1°C. It is not certain that the thermistor did not perturb the field or create localised "hot spots" of energy deposition. The 2450 MHz microwave exposure was pulsed (50 Hz, duty cycle 1:3) with a nominal SAR of 75 W/kg. While the paper reports an increase in percentage of chromosome aberrations and of micronuclei there is no effect on the rate of sister chromatid exchanges. Cell cycle kinetics was unchanged as would be expected if temperature was unchanged.

In a recent presentation by this group (Verschaeye et al 1994) at the BEMS conference, they reported effects from mobile telephones in 32 subjects exposed to 450 or 954 MHz. Chromosome aberrations were assayed by changes in DNA electrophoresis. In addition, rats exposed to 954 MHz showed a statistically significant increase (≈25%) in the SCE frequency when microwave radiation accompanied application of the mutagen, mitomycin C. Evidence of a synergistic association is presented. However, the details of this work were very sketchy, with very small populations and little useful information on dosimetry, (no SAR values given on exposure duration). The data appeared to be sparse and the authors reported it as "preliminary". The obvious question then is; how does the acceptance of such material at an international conference assist the development of scientific knowledge? Previous studies using SCE as an

endpoint have failed to demonstrate an effect of microwave radiation or a synergistic effect when applied together with known mutagens (Meltz et al 1989, Meltz 1991).

Concerns about this paper relate to the dosimetry and the population size and relevance of the statistical analysis. A result is published on the basis of two blood samples from two donors. Groups of different sizes are compared. In fact the data is weighted in one experiment where a control group of 500 was compared with exposed groups of 200 and the aberrations expressed as a percentage. The fact that only 100 cells could be examined in the third group suggests a problem in the culture protocol. The data is rather weak, and the authors state that; "This work must be considered as a preliminary pilot experiment ... ...". As far as implications to human health are concerned, there is little that can be inferred from this study.

Although it was not the intent to criticise individual scientific publications this may be taken as representative of some of the peer-reviewed literature. There were almost 100 papers published in the Bioelectromagnetics journal in 1993. One has to wonder about the peer-review process that allows publication of preliminary work-in-progress standard of manuscript as scientific papers. Perhaps a more restrictive policy would limit the otherwise vast number of publications in the EMR field, many of which contribute little other than adding to the confusion and fuelling the desire for more independent reviews. The situation is further exacerbated by the apparently unconditional acceptance of every abstract submitted to the BEMS annual conferences. As a result the 1994 conference yields 198 abstracts and 222 posters.

Another study has addressed the issue of possible long-term effects of RF exposure on lymphocyte chromosome integrity in an occupationally exposed group (Garson et al 1991). The endpoint involved chromosome breakages and compared a high risk group of radio-linemen with a control group from office workers in the telecommunications industry. The exposed group received radiation over the range 400 kHz to 20 GHz at or below the safety standard for occupational exposure. An assay for chromatid gaps and breaks, chromosome breaks and "other" aberrations found no statistically significant difference between the exposed and control groups. A requirement of the study was that all subjects had worked for at least 5 out of the previous 6 years as radio-linesmen, and the last exposure was no more than 12 months before the study. The

lymphocytes were obtained from the peripheral blood circulation. Although this is an acceptable protocol for chromosome breaks, it might have been more desirable to have used an exposed group comprised entirely of people who were exposed to RF radiation at the time of the assay rather than up to 12 months before.

The information from this paper supports the commonly accepted view that there is no evidence that chronic exposure to low level RF radiation has clastogenic effects.

Recent reports contrary to that opinion were presented at the 16th conference of the BEMS (June 1994). In one report on the health effects of radar exposures on workers an increased incidence in aberrations and micronuclei was reported (Garaj-Vrhovac & Fucic 1994). The study group comprised 40 workers employed in antenna maintenance occupations for a mean of 12.5 years (range from 0.5 to 26 years). Peripheral blood lymphocytes were reported to have significantly higher incidence of structural aberrations and micronuclei in the exposed group (8.2 - 26 GHz, SAR mostly 5 mW/cm^2 up to a maximum value of 26 mW/cm^2). These authors have previously published findings that microwave exposure is clastogenic and that it produces an increase in the number of micronuclei in human lymphocytes following *in vivo* exposure (Fucic et al 1992) in the workplace to pulsed microwave radiation in the range 1.25 to 1.35 GHz at power densities 0.01 to 20 mW/cm^2. They also reported an increase in aberrations including chromosome and chromatid breaks, acentrics and dicentrics following *in vitro* (Garaj-Vrhovac et al 1992) exposure at 7.7 GHz. Increased rates of aberrations were reported for power densities from 0.5 to 30 mW/cm^2 for 10 to 60 min. It is uncertain why these studies should contain such a high degree of sensitivity.

7.0 HUMAN BIOEFFECTS

SUMMARY

People with normal hearing are able to perceive pulse-modulated RF and microwave radiation between about 200 MHz and 6.5 GHz. The sound perception probably results from the thermoelastic expansion of brain tissue caused by a small but rapid increase in temperature. The perception threshold for pulses shorter than 30 μs depends on the specific energy density in the head and has been estimated to be as low as 30 mJ/kg. Receptors in the skin are sensitive and absorb RF and microwave radiation at power densities of approximately 300 W/m^2 at 3 GHz during exposure for 10 s. Meanwhile infrared radiation applied for 10 s is detected at power densities an order of magnitude lower due to its greater absorption (therefore greater SAR) in the skin. Thresholds of perception depend on frequency and exposure duration as well as the locality on the body and the exposed area. The perception of skin warming by microwave and RF frequencies in the rage 0.5-100 GHz does not afford a reliable means of protection against potentially harmful exposure from heating (Elder 1984a).

Healthy subjects at rest in light clothing and in comfortable ambient conditions (21 - 22°C, 50% RH and adequate ventilation) are able to dissipate RF power at SARs of 1 W/kg, and to up to 4 W/kg for short periods, although sweating and increased heart rate was observed in the upper part of this range after 20 min exposure. The total heat load of an exposed person represents the sum of the SAR from RF or microwave heating and the rate of metabolic heat production and must be compensated by heat loss. The limit of tolerable SAR is affected by adverse conditions (high temperatures or humidity), moderate physical exercise, some medication, or conditions which impair thermoregulation (including pregnancy). The relationship between local SAR and body temperature increase is not well established. Neither is the rise in local temperature in response to high, localised SARs elsewhere within the body. Further dosimetric research is required to determine whether local heating, rather than whole-body heating, could become a limiting factor in some circumstances.

7.1 Perception

Auditory perception

It is well established that humans with normal hearing are able to perceive pulse-modulated RF and microwave radiation as buzzing, clicking, hissing, or popping noise, depending on the modulation characteristics (NCRP 1986). First reports (Frey 1961, 1962, 1963) of this phenomenon described the perception of pulsed radiation frequencies between 216 MHz and 2.98 GHz. Pulse widths varied between 1 and 1000 μs and the average threshold power density was 4 W/m^2. Sensitivity was increased by lowering the ambient audible noise levels. Since then the average power density threshold for RF hearing has been reported as low as 0.01 W/m^2 in people with normal hearing (Cain & Rissman 1978). Perception of different pulse-modulated frequencies has been reported (Constant 1967) at 3 and 6.5 GHz but not at 9 GHz.

In a study of the threshold conditions for this effect in one human subject exposed to pulsed 2.45 GHz radiation (Guy et al 1975) it was determined that, for pulse widths less than 30 μs, the perception threshold depended on the energy density per pulse. A threshold value of 280 mJ/m^2 (estimated 10 mJ/kg) was measured when the subject wore earplugs. An animal study measured brain stem auditory evoked potentials in guinea-pigs exposed to pulsed 918 MHz radiation (Chou & Guy 1979) and concluded that the threshold for microwave hearing is related to the incident energy density per pulse for pulses shorter than 30 μs and is related to the peak power for longer pulses (up to 500 μs).

It is generally agreed (NCRP 1986; Foster & Finch 1974) that the mechanism of acoustic perception of short pulses of RF and microwave radiation is due to thermoelastic expansion of brain tissue following a small but rapid temperature increase ($<10^{-5}$°C). As the effect must depend on absorption of some incident energy it would be limited to frequencies which penetrate the skull and are significantly absorbed by brain tissue. The effect of the expansion is an acoustic pressure wave which is transmitted through the skull to the cochlea where vibration-sensitive hair cell receptors respond as they would to acoustically generated pressure stimuli. It has been calculated that the frequency of the induced sound is related to head size and the acoustic properties of brain tissue, regardless of the RF frequency (Lin 1977).

Cutaneous perception

The absorption of RF and microwave radiation can be detected by receptors in the skin, although variability in the sensitivity has been reported. Most studies have involved exposures in the frequency range 2.45 - 10 GHz (NCRP 1986; Elder 1984; Adair 1983). Microwave radiation was found to be ten to fifteen times less effective than infrared in heating the skin (Justesen et al 1982). The difference is attributed to the scattering of two-thirds of the microwave energy and the relatively small proportion of microwave energy, estimated to be one-fifth of the value for infrared radiation, absorbed in the skin. Subjective awareness of warmth is not a reliable indicator of microwave hazard. Perception threshold values are frequency dependent. Threshold response by different parts of the body is variable, at low levels of irradiation the face is the most sensitive region, the trunk intermediate, and the limbs least sensitive (by a factor of two or three). Threshold power density must be qualified by the duration of the stimulus and the area of the exposed skin; e.g. infrared power density thresholds decrease as the duration of the stimulus increases (up to a critical value), and as the size of the area of exposed skin increases. The response latency is also an important variable that depends on stimulus duration and area. In general, skin sensory receptors respond to transient rather than constant stimuli, although the effect of adaptation on the perception of microwave radiation is not known.

Consensus of a panel of a Symposium on Microwaves and Thermoregulation, Connecticut, 1981 was that microwaves of 30 GHz and above would probably be similar to infrared in their perception threshold values and may be sensed at the limit recommended by the American National Standards Institute (ANSI 1982) which is 50 W/m^2 in this part of the spectrum (Adair 1983). However, over much of the radiofrequency spectrum, the perception thresholds are higher than the ANSI standard, and the deeper penetration results in a larger mass of tissue heated by microwaves for a comparable rise in skin temperature. A further problem is that the threshold temperature (41-42°C) of cellular injury for sustained temperature elevations is below the threshold (= 45°C) of pain. For the frequency range 0.5 MHz to 100 GHz, cutaneous perception of heat and thermal pain may be an unreliable sensory protection mechanism against RF radiation exposures.

7.2 Thermophysiological responses

Normal body temperature is maintained by a complex control system of heat loss or gain responses, including behaviour (Simon et al 1986), at a so-called "set-point" value of around 37°C. Body temperature is maintained within a narrow range (=0.5 °C fluctuation) by the processes of sweating or increasing metabolic heat production. Data on thermoregulatory responses to RF or microwave heating is obtained from experiments with passively heated volunteers. Ambient conditions such as high humidity can profoundly limit the thermoregulatory capabilities and exert a significant effect on the ability to tolerate different whole-body SARs. A change in ambient temperature of 1°C can produce a change in heat flux of about 0.15 W/kg in a clothed individual. It is clear then, that the thermal burden from a given SAR that can be tolerated in a cool, dry environment may pose a health hazard if the environment is either very hot or humid.

In an attempt to estimate the maximum SAR that could be tolerated by a fit healthy person, under strictly specified ambient conditions, Durney et al (1978) defined a rectal temperature of 39.2°C as an upper limit of physiologically tolerable body temperature. A SAR of approximately 3 W/kg was calculated to induce this rectal temperature within 1 h in a person in an environmental temperature of 40°C and a relatively humidity of 80%. Raising the ambient temperature to 41°C decreased the tolerable SAR to about 1 W/kg. The rectal temperature of 39.2°C is an estimate of the upper limit of tolerance and should not be considered as a safety limit as there is a wide range in physiological tolerance amongst different members of the population.

Other physical and physiological factors which reduce the ability to adapt to an extra heat load include old age, obesity, hypertension and effects of many drugs such as diuretics, antihistamines, tranquilizers B-blockers and amphetamines. The thermoregulatory ability of infants is not well developed while pregnant women have an extra circulatory load which may compromise their ability to dissipate heat. Heat loss from the embryo and fetus across the placental barrier may be less efficient than heat dissipation in other, well-vascularised tissues.

The American National Institute of Occupational Safety and Health (NIOSH 1972) and the American Conference of Governmental Industrial Hygienists (ACGIH 1983) recommend an upper threshold limit value of a rise in body temperature of 1°C. This is endorsed by the World Health Organisation (WHO

1980). It was recommended that rates of physical work and environmental factors should be such as to limit excursions of body temperature beyond 1°C.

Calculations relating whole-body SAR to increases in body temperature are generally supported by the results of studies of the thermoregulatory responses of patients and volunteers exposed to RF fields of up to 4 W/kg in magnetic resonance systems. However, the subjects are exposed at rest and in controlled environments and the whole-body SARs quoted result from much higher, localised, SARs.

Shellock et al (1989) exposed the abdomen of six volunteers to 64 MHz RF magnetic fields for 30 min at whole-body SARs from 2.7 to 4.0 W/kg. Although body temperature was reported to rise by an average of only 0.1°C, all of the subjects reported feeling warm and had visible signs of perspiration on their forehead, chest and abdomen during the procedure. Abart et al (1989) reported increases in rectal or sublingual temperature of up to 0.7°C, with a mean increase of 0.33°C, in 12 healthy volunteers exposed at a whole-body SAR of 3 W/kg for 20 min. Heart rate was also observed to increase by up to 45%. The magnitude of body temperature increase was similar to that reported in 12 healthy volunteers exposed at a whole-body SAR of 4 W/kg for 20 min (Schaefer et al 1985).

In contrast, differing thermoregulatory responses (to magnetic resonance imaging) have been reported in 50 patients with unspecified clinically impaired temperature regulation (Shellock & Crues 1987). Exposure to 64 MHz RF magnetic fields at whole-body SARs between 0.4 and 1.2 W/kg for 15 min increased body temperature by 0.5°C at low SARs. The mean skin temperatures of localised areas of the hand and trunk when imaged, were reported to increase by up to 1.2°C. Another study (Kido et al 1985) reported mean increases in body temperature of 0.5°C in volunteers exposed to a magnetic resonance abdominal scan for 17 min where the whole-body SAR was only 0.8 W/kg. Heart rate increased by 3 beats per minute.

Calculations of SAR distribution based on a heterogeneous model of the human body indicate that localised SARs in small tissue volumes could be 10 to 70 times greater than the whole-body average SAR during exposure to magnetic resonance imaging (Orcutt & Gandhi 1988).

The relationship between local SAR and temperature increase is not well established. Localised heating may occur in various parts of the body depending on the conditions of exposure, particularly antenna proximity and the radiation

wave-length, and on the shape and variation in tissue conductivity and blood circulation in the exposed part of the body. The amount by which localised heating will exceed whole-body average is not known at present.

8.0 EFFECTS OF EMR ON CENTRAL NERVOUS SYSTEM

SUMMARY

From studies on human perception it is accepted that very low levels of microwave exposure elicit a response, known as microwave hearing, that is thought to be due to a thermoelastic change producing a pressure wave in the brain and auditory sensory apparatus. It would not be too surprising to find associated transitory changes in the electrocortical activity, although the results in humans and animals are equivocal probably due to artefacts in experimental technique. There have been contradictory reports (by the same group of researchers) on the effect of microwave radiation on the permeability of the blood-brain barrier, making sensible interpretation rather difficult.

Changes in animal behaviour induced by high exposures (SAR > 4 W/kg) create a significant increase in body temperature and would, therefore, invoke a response in the hypothalamus and adrenal corticosteroids. Other sensitive endocrine organs such as the pituitary and pineal glands would also respond to such a gross physical insult.

Of more interest from a human health perspective are the reports of impaired learning and memory function in rats following a exposure to relatively low level SAR 0.6 W/kg. The effect has been shown to be due to microwave-induced activation of brain opiod activity. Such subtle neurophysiological responses are of particular interest. Alterations in DNA arrangement have also been detected by sensitive electrophoretic tests, following exposure to similarly low SAR. This work urgently needs verification and extension.

Introduction

Mammalian Blood -Brain Barrier

The blood brain barrier in the choroid plexus separates the brain and cerebal spinal fluid of the central nervous system from the blood (and potential blood-borne toxins or micro-organisms). The barrier consists of specialised capillaries, the cells of which form "tight junctions" in an essentially continuous layer. In contrast to most other capillaries in the body those in the cranial vault lack intracellular fenestrae that allow passage of small molecules from blood to the interstitial fluid. The pinocytotic vesicles that transport large molecules across capillaries in peripheral organs are also rare in the capillaries in the brain.

Functionally, the blood-brain barrier is a selectively permeable hydrophobic membrane that allows the passage of small lipid-soluble molecules. Lipid-insoluble substances encounter regulatory interfaces between the blood and the Central Nervous System that control their transport. Certain lipid-insoluble molecules, such as glucose, cross the membrane via carrier proteins.

Electrophysiological Responses

Since the central nervous system co-ordinates and controls an organism's responses to its environment through autonomic and voluntary movements and neurohumoral function, any effect of radiofrequency radiation is important. The reaction of the central nervous system to microwaves may provide evidence of early disturbance in regulatory function of many systems. For instance, the hypothalamus of the forebrain controls thermoregulation and secretion of hormones, while the hippocampus serves behavioural functions such as memory and emotion. Information is generally passed from one neuron to another via the release of neurotransmitter chemicals, such as acetylcholine, dopamine, serotonin, γ-amino-butyric-acid (GABA) or endogenous opiods. Binding of the neurotransmitter to a receptor triggers a series of reactions that affect the post-synaptic cell. Many drugs exert their effects by binding to the receptors. Those which activate the receptor are known as agonists while those which block the action of the endogenous neurotransmitters are antagonists. Since changes in receptor properties can last for many days an animal's normal physiological functions can be altered by any interference in the neurotransmission pathway.

Experimental Evidence

8.1. *Blood Brain Barrier*

Initial work suggested that exposure to low level pulsed microwave radiation significantly affected blood-brain barrier permeability. Later workers attempted to confirm and extend these observations. The subject has been previously reviewed (Blackwell & Saunders 1986; NCRP 1986). However, the interpretation of the results is difficult; some of the evidence is contradictory and many of the results may well have been confounded by various factors such as the use of anaesthesia or the difficulty in either removing or estimating the amount of tracer. The effects of microwave radiation on the permeability of the blood-brain barrier have been investigated by tracing the penetration, after intravenous injection, of labelled compounds such as protein-bound dyes (fluorescein), radiolabelled saccharides, or horseradish peroxidase. Frey et al (1975) reported

the penetration by fluorescein in anaesthetised rats after irradiation at low levels (SAR = 0.04 - 0.5 W/kg) of pulsed or continuous microwave radiation. In a replication of this study (Merritt et al 1978) and another that exposed conscious rats to 2.45 GHz radiation (Williams et al 1984) at up to 13 W/kg the fluorescein content in brain tissue was found to increase with increasing microwave exposure and brain temperature. However, a decrease in renal clearance of fluorescein was also observed in animals which were hyperthermic (> 41°C) suggesting that this may have accounted for the elevation of brain tissue values. The same exposure produced opposite results on the transport of horseradish peroxidase across blood vessel endothelial cells in the brain tissue in conscious animals (Williams et al 1984). The histological assay is less susceptible to artefacts than measurement of fluctuating plasma levels, but is more difficult to quantify. An increase in peroxidase uptake was reported in conscious Chinese hamsters exposed for 2 or 8 h to 100 W/m^2 (SAR estimated = 2 - 3 W/kg) at 2.45 GHz. This effect was confirmed and shown to be reversible (Albert & Kerns 1981).

A number of authors have looked at the uptake into brain tissue of radiolabelled saccharides. The exposure of anaesthetised rats for 20 min to low levels (3 - 20 W/m^2; SARs estimated to be 0.06 - 0.4 W/kg) of pulsed 1.3 GHz microwave radiation was reported to increase significantly the permeability of the blood-brain barrier to ^{14}C-labelled saccharides compared to the permeability to ^{3}H-labelled water. However, when brain tissue concentrations of ^{14}C-labelled saccharides were compared to circulating plasma levels in exposed and sham-exposed animals no change in the uptake of sucrose or inulin in the brain tissue was found in anaesthetised rats exposed to continuous or pulsed 1.7 GHz radiation at an SAR of 0.1 W/kg (Ward & Ali 1985). Previous experiments reported a decrease in the uptake of sucrose into the brain tissue of anaesthetised and conscious rats exposed for up to 90 min, to 2.45 GHz radiation at SARs of 0.1 - 13 W/kg. Microwave exposure at SARs up to 6 W/kg increased permeability to sucrose but not inulin in anaesthetised rats, while sucrose permeability in conscious rats was unaffected by microwave exposure (NRPB 1993).

In a study on the effects of MRI exposure on the blood-brain barrier of the rat Salford et al (1992) reported leakage of Evans-blue stained proteins. In a subsequent study using unanaesthetised rats exposed to 915 MHz c.w. or pulsed (modulated at 200, 50, 16 or 8 Hz) they reported passage of albumin across the blood-brain barrier (Salford et al 1993). There was no significant difference between c.w. and pulsed exposures. Dosimetry information was sparse but it

appears that the extravasation effect was observed to a varying extent at SAR from 0.33 to 3.3 W/kg.

8.2 *Electrophysiological Responses*

Changes in the function of nervous tissue, measured electrophysiologically, have been reported during or after whole-body or localised irradiation. These studies are prone to measurement artefact since the use of metallic recording electrodes can greatly perturb the applied field, causing enhanced energy absorption, and there is a chance of field-induced pickup in the leads and electrodes.

High levels of microwave exposure have produced decreases in the latency of evoked potentials recorded during exposure. Johnson and Guy (1972) exposed the heads of cats to 918 MHz radiation for 15 min at 10 - 400 W/m^2 and recorded decreased latency in the evoked potentials in the thalamus above a SAR threshold of 2.5 - 5 W/kg. Similar responses were also achieved by conventional heating of the thalamus, and microwave-induced effects could be prevented or reversed with concurrent brain cooling (NRPB 1993).

A series of well-conducted experiments by Lai and colleagues (Lai et al 1984, 1987, 1988, 1989) has shown that exposure to low level pulsed and continuous microwave radiation can act as a non-specific stressor. The acute exposure of rats to pulsed 2.45 GHz radiation (2 μs pulses at 500 pps) at 10 W/m^2 (a whole-body average SAR 0.6 W/kg, with a peak SAR of 600 W/kg) was shown to affect the activity of cholinergic neurons in the forebrain. The threshold was reported as 0.45 W/kg, corresponding to a specific energy per pulse of 0.9 mJ/kg (Lai et al 1989). The relative effectiveness of pulsed and continuous microwave radiation at a whole-body SAR of 0.6 W/kg varied in different regions of the brain (Lai et al 1988). Rats exposed for 45 min immediately before daily training sessions in a radial arm maze showed delayed learning performance (Lai et al 1988, 1989).

The radial-arm maze test was used to demonstrate impaired short-term memory function following an acute exposure of 45 min to 2.45 GHz RF at power density of 1 mW/cm^2 and estimated whole body SAR 0.6 W/kg. The mechanism of effect has been proposed as one of activation of endogenous opiods in the brain resulting in decreased cholinergic activity in the hippocampus (learning centre). In addition, DNA in brain cells was reported to be damaged, assayed by electrophoretic techniques following a single 45 min exposure (Lai, private communication). The effects were observed with both pulsed (2μs, 500 pps) and

c.w. waveforms. Breakage of DNA in the CNS and testes has also been reported recently (Sarka et al 1994) using the same sensitive electrophoretic technique, following exposure to microwaves at 1.18 W/kg SAR.

It is vital that these studies are verified by independent laboratories. The induced changes to neural DNA are unexpected, particularly at the exposure levels used in these studies.